

extracellular domain (ECD) of CaSR with various spectroscopic methods including the tryptophan fluorescence titration, circular dichroism (CD) and nuclear magnetic resonance (NMR) as well as molecular dynamic simulation. These results provide important implications for our understanding of how the CaSR integrates information about these two completely different classes of agonists—an inorganic divalent cation, and another hand, a nutrient—how the receptor senses these agonists in healthy and diseased states.

#### 2069-Pos Board B88

##### Synthetic Demethylwedolactones Derivatives Inhibit Invasive Growth of Mda-Mb-231 Breast Cancer Cells *In Vitro* and *In Vivo*

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Combretastatins, which are an important group of anticancer drugs, were isolated by Pettit et al. from the African tree *Combretum caffer* in 1989. Additionally, Liang et al. have reported that ten coumestans were isolated from the roots of *Hedysarum multijugum*, which is a plant in *Hedysarum* Linn. of the family *Leguminosae* used as a folk herbal drug in northwest China. Coumestans comprise a class of naturally occurring products with a variety of biological activities including phytoestrogenic, antibacterial, antifungal, antimyotoxic, and phytoalexin effects. The anticancer properties of demethylwedolactone (DWEL) and wedolactone (WEL), which are naturally occurring coumestans, have not been well characterized. Due to their biological activities, the synthesis of DWEL is achieved in which the longest linear sequence is only eight steps in 38% overall yield from commercially available phloroglucinol. Furthermore, the molecular model was examined the interactions of proteins and ligands as well. Finally, in this study, we investigated the anti-invasive effects of synthetic WEL and DWEL on human MDA-MB-231 breast cancer cells. We found that WEL and DWEL inhibited the anchorage-independent growth and also suppressed cell motility and cell invasion of MDA-MB-231 cells. In addition, WEL and DWEL reduced the activity and expression of matrix metalloproteinases (MMPs) involved in blocking the I $\kappa$ B- $\alpha$ /NF $\kappa$ B and MEK/ERK signaling pathways in MDA-MB-231 cells. Furthermore, DWEL suppressed the metastasis and lung colonization of the tumor cells in the nude mice. Altogether, these data suggest that DWEL derivatives exert anti-invasive growth effect on breast cancer cells.

#### 2070-Pos Board B89

##### Molecular Dynamics of DOT1L and Modeling of EZH2

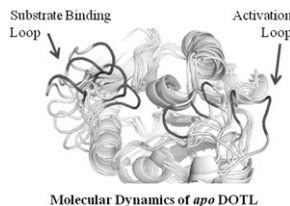
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Histone methyltransferases are enzymes that modify histone proteins via methylation of lysine or arginine residues. These epigenetic modifiers, such as DOT1L and EZH2, have been found to play important roles in leukemogenic processes.

Crystallographic and docking methods studied interactions within the DOT1L binding site. Crystal structures of DOT1L also demonstrated variance in the binding mode of ligands, possibly due to rearrangement in the DOT1L binding site. We investigated this possibility by molecular dynamics (MD) simulations and confirmed significant rearrangement of the substrate binding and activation loops of DOT1L upon binding to competitive inhibitors. The druggability and volume fluctuations of the binding pocket over time were determined.

The EZH2 methyltransferase is of interest due to its aberrant activity in many cancers. Because there is no published crystal structure of EZH2, we used homology modeling with homologous proteins as templates. This model provides structural information regarding the binding modes of the S-adenosyl methionine (SAM) cofactor and potential inhibitors of EZH2. Through the synergistic combination of *in silico* drug design, organic synthesis and biochemical assays, our modeling efforts for DOT1L and EZH2 will guide the chemical syntheses of potent and selective inhibitors of these enzymes.



#### 2071-Pos Board B90

##### T Cell Receptor Specificity, Cross-Reactivity, and MHC Restriction are Inextricably Linked and Result from Cooperative Engagement of the Composite Peptide/MHC Surface

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T cell receptors (TCRs) recognize peptides bound and presented by major histocompatibility complex (MHC) proteins using multiple complementarity determining region (CDR) loops. While numerous analyses have illuminated

structural and biophysical aspects of TCR recognition, how the distribution of binding free energy within TCR-pMHC interfaces promotes unique TCR recognition features, including MHC restriction and the apparent dichotomy of specificity and cross-reactivity, remains unclear. Utilizing double mutant cycles, here we performed a comprehensive structural and thermodynamic deconstruction of the interaction between the A6 TCR and the Tax peptide presented by the class I MHC HLA-A2. In contrast with general expectations, we observed that the central regions of the peptide and its interactions with the hypervariable CDR3 loops contribute little to specificity, instead promoting by dynamic effects the cross-reactivity that is a hallmark of TCR recognition. We also observed that TCR restriction towards HLA-A2 results from not from conserved interactions with the germline loops, but instead from strong interactions with the hypervariable CDR3 loop of the  $\alpha$  chain. Formation of these latter interactions, however, is dependent upon the unique structural properties of the peptide, highlighting that TCR specificity towards peptide and MHC can emerge from the need to engage a unique, composite peptide/MHC interface with tightly coupled structural properties.

#### 2072-Pos Board B91

##### Dissecting Signal Control in the Multidrug Sensor, BMR

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Multidrug (MD) (or xenobiotic) eflux actively removes cytotoxic chemicals from the interiors of normal-functioning cells. However, high levels of eflux can render drug-targeted cells resistant to a broad-range of therapeutic agents, including those to which cells were never exposed. Key multidrug resistance (MDR) contributors include allosteric eflux pumps, gene regulators and other sensory systems that mediate the detection and extrusion of diverse drugs from cellular environments. To date, MDR functions remain only partially understood. Ligand-dependent allosteric control in BmrR has been quantitatively addressed using *in vitro* transcription experiments, dose-response curves and thermodynamic models that relate the observed transcriptional responses to ligand binding and changes in BmrR conformation. Preliminary results indicate that allosteric control in BmrR is sensitive to both energetic and structural aspects of ligand recognition. Importantly, increased cooperativity in signal control relative to recognition implicates a major allosteric role for the RNA polymerase.

#### 2073-Pos Board B92

##### Fragment-Based Approach Identifies a Novel Inhibitory Site on DHPS

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Dihydropteroate synthase (DHPS) is an essential enzyme in the bacterial folate biosynthetic pathway. It catalyzes the condensation of 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPP) with p-aminobenzoic acid (pABA) to form the folate intermediate, 7,8-dihydropteroate. DHPS is the target of the sulfonamide class of antibiotics. Widespread resistance to sulfonamides has decreased their clinical use. The active site of DHPS is comprised of three sub-sites: the structured "pterin" site, the flexible pABA site, and the anion binding pocket. Most of the drug resistant mutations have been mapped to the pABA sub-site of DHPS. Using an NMR ligand-based screening approach, a number of structurally unrelated fragment-like small molecules have been identified that inhibit the enzymatic activity of DHPS from *Bacillus anthracis* (Ba), *Yersinia pestis* (Yp), and *Staphylococcus aureus* (Sa). Fragment hits were shown to target the three sub-pockets of the active site and a novel site distinct from the active site. The latter site potentially inhibits via an allosteric mechanism and has been characterized by high resolution X-ray crystallography.

We screened the Maybridge<sup>®</sup> Fragment library of 1,100 fragments using water ligand observed gradient spectroscopy (waterLOGSY) as a primary screen which resulted in a hit rate of 6.7%. Of the 74 hits, 25 were shown to inhibit DHPS activity using two independent enzyme activity assays. A total of eight compounds inhibited the activity of DHPS from three different species (Ba, Yp, and Sa). In addition to screening for inhibition, the fragment hits were validated using a number of biophysical techniques including 2D NMR, SPR, competition waterLOGSY, and X-ray crystallography. Herein, we focus on two fragment hits for which high-resolution x-ray crystal structures are available.

#### 2074-Pos Board B93

##### Interactomics of Blebbistatin

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Photoreactive molecules like aryl azides play important role in life sciences, as practical tools to achieve precisely timed covalent cross-links between ligands and targets. Since the azido group is small, stable and biologically inert,